Mineralisation of atrazine, metolachlor and their respective metabolites in vegetated filter strip and cultivated soil

Larry J Krutz,[1]* Terry J Gentry,[2]† Scott A Senseman,[3] Ian L Pepper[2] and Dennis P Tierney[4]

[1]*USDA-ARS, Southern Weed Science Research Unit, PO Box 350, Stoneville, MS 38776, USA*
[2]*Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, AZ 85721, USA*
[3]*Department of Soil and Crop Sciences, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843, USA*
[4]*Environmental Stewardship and Regulatory Policy, Syngenta Crop Protection, Greensboro, NC 27409, USA*

Abstract: In vegetated filter strips (VFS) the presence of perennial vegetation, rhizodeposition of labile organic substrates and the accumulation of an organic residue thatch layer may enhance microbial numbers and activity, thereby increasing the potential for mineralisation of herbicides and herbicide metabolites retained during run-off events. The objective of this laboratory experiment was to compare the mineralisation of atrazine and metolachlor with that of their respective metabolites in VFS and cultivated soil. With the exception of total bacteria, propagule density of the microbial groups, endogenous soil enzymes and microbial diversity were higher in the VFS soil. This correlated with increased mineralisation of metolachlor and its metabolites in the VFS soil and indicates potential for VFS to curtail the subsequent transport of these compounds. In contrast, the mineralisation of atrazine and the majority of its metabolites was substantially reduced in VFS soil relative to cultivated soil. Consequently, the potential for subsequent transport of atrazine and many of its metabolites may be greater in VFS soil than in cultivated soil if reduced mineralisation is not offset by increased sorption in the VFS.

Keywords: pesticide; metabolite; mineralisation; buffer strip; transport; microbial activity

1 INTRODUCTION

Atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) and metolachlor (2-chloro-6′-ethyl-*N*-(2-methoxy-1-methylethyl)aceto-*o*-toluidide) are applied pre-emergence and post-emergence for weed control in a variety of crops.[1] Atrazine controls primarily broadleaf weeds in corn, *Zea mays* L., grain sorghum, *Sorghum bicolor* (L.) Moench, and sugarcane, *Saccharum officinarum* L., while metolachlor is applied for grass control in corn and cotton, *Gossypium hirsutum* L. In soil, these herbicides are subject to microbial and chemical degradation. Primary degradation products of atrazine include desethylatrazine (6-chloro-N^4-isopropyl-1,3,5-triazine-2,4-diamine, DEA), deisopropylatrazine (6-chloro-N^2-ethyl-1,3,5-triazine-2,4-diamine, DIA) and hydroxyatrazine (6-hydroxy-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine, HA) (Fig. 1).[2] Formation of DEA and DIA from atrazine occurs through *N*-dealkylation, a microbially mediated process.[3,4] Formation of HA from atrazine occurs through both biological and non-biological hydrolytic pathways.[5,6] Degradation products of metolachlor include metolachlor ethanesulfonic acid (2-[6-ethyl-*N*-(2-methoxy-1-methylethyl)-*o*-toluidino]-2-oxoethanesulfonic acid, ESA) and metolachlor oxanilic acid (2-[6-ethyl-*N*-(2-methoxy-1-methylethyl)-*o*-toluidino]-2-oxoacetic acid, OA) (Fig. 1).[7–9] The formation of ESA from metolachlor arises from glutathione conjugation, a common detoxification process for several organisms.[10,11] Pathways describing the degradation of metolachlor to OA have not been published.

Despite the potential for degradation, atrazine, metolachlor and their respective metabolites have been detected in surface waters throughout the USA. The maximum concentration in 95 Midwestern streams was 136 μg litre^{-1} for atrazine, 7.5 μg litre^{-1} for DEA, 7.4 μg litre^{-1} for DIA and 3.7 μg litre^{-1} for HA.[12] Similar values are reported for the lower Mississippi River, various Midwestern streams and the playa lakes of West Texas.[13–16] Likewise, the median concentration in 12 stream sites located in eastern Iowa was 0.15 μg litre^{-1} for metolachlor, 3.0 μg litre^{-1} for ESA and 0.7 μg litre^{-1} for OA.[17] Similar concentrations are reported for the playa lakes of West Texas.[16] Consequently, means for limiting the transport of atrazine, metolachlor and their respective metabolites are desirable.

Vegetated filter strips (VFS) are narrow strips of permanent vegetation planted adjacent to cropland with the intent to reduce herbicide transport

* Correspondence to: Larry J Krutz, USDA-ARS, Southern Weed Science Research Unit, PO Box 350, Stoneville, MS 38776, USA
E-mail: jkrutz@ars.usda.gov
†Current address: Department of Soil and Crop Sciences, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843, USA
(*Received 22 June 2005; revised version received 15 November 2005; accepted 21 November 2005*)
Published online 12 April 2006; DOI: 10.1002/ps.1193

 Pest Manag Sci 1526–498X/2006/$30.00

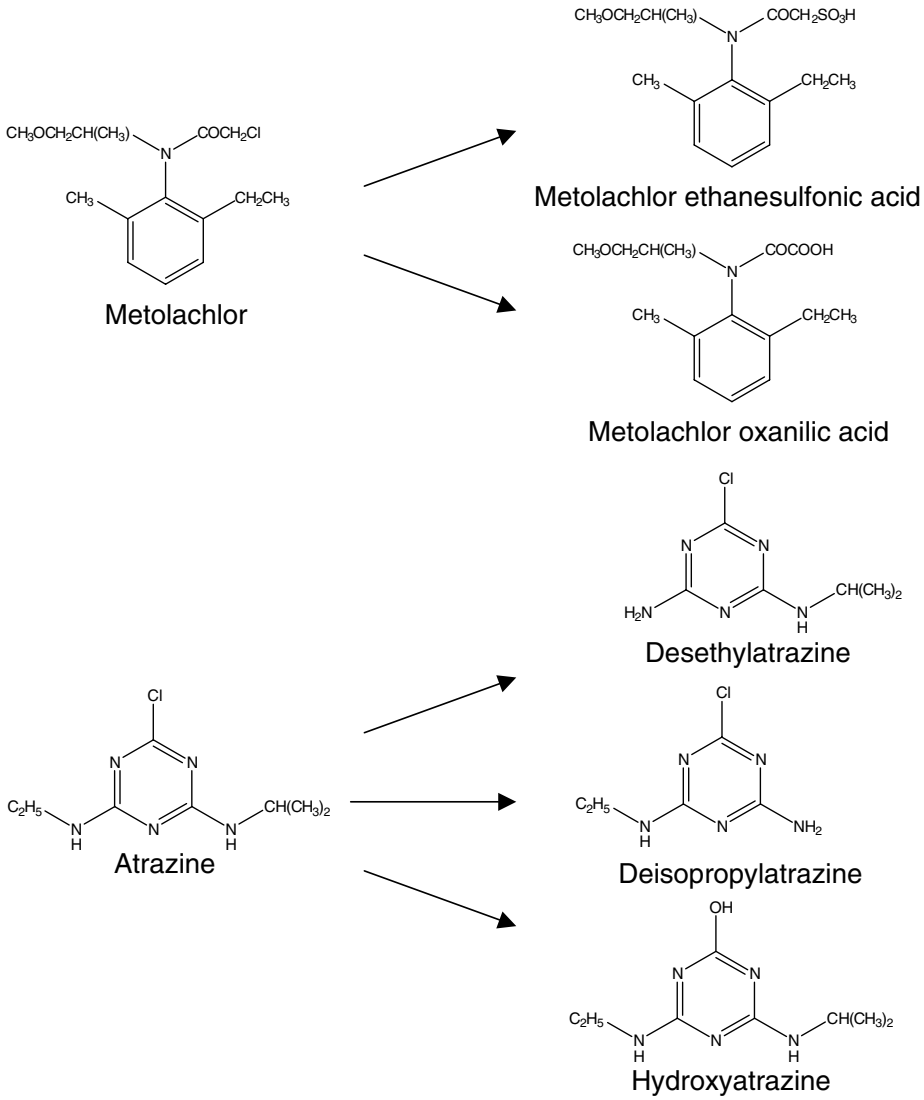

Figure 1. Structures of metolachlor, atrazine and metabolisation products.

from agricultural application zones. By design, VFS accumulate greater above- and below-ground organic matter than adjacent cultivated soil.[18–23] The accumulation of organic carbon (OC) in VFS soil is significant in that it is correlated with the sorption of non-polar and weakly basic herbicides. Greater sorption of atrazine, HA, metolachlor, fluometuron and isoproturon has been reported for VFS soil compared with cultivated soil.[18–23] In contrast, sorption values for the ionic metabolites of metolachlor, ESA and OA, and the *N*-dealkylated metabolites of atrazine, DIA and DEA, were not different between VFS soil and cultivated soil.[22,23] These data indicate that the mobility of non-polar and/or weakly basic herbicides may be reduced in VFS soil owing to their affinity for soil OC, while the mobility of ionic and/or relatively polar species will likely be similar between soils, since factors other than organic carbon dictate their sorption. Consequently, for VFS to effectively reduce the transport of ionic and/or relatively polar species, the microclimate created by the VFS must be amenable to the mineralisation and/or degradation of these compounds.

Higher microbial numbers, microbial activity and soil enzymatic activity have been reported for VFS soil compared with cultivated soil.[18,21] These data suggest that the presence of perennial vegetation, rhizodeposition of labile organic substrates and the accumulation of an organic residue thatch layer enhance soil microflora and their metabolic activity. Moreover, the enriched density of several classes of soil microflora and indices of microbial activity in VFS soil indicate potential for enhanced degradation of herbicides. Greater degradation of metolachlor, fluometuron, atrazine and isoproturon has been reported in VFS soil than in cultivated soil.[18,21,24–26] To date, degradation and/or mineralisation of herbicide metabolites in VFS soil have not been evaluated.

In our previous work, field studies indicated that buffalo grass, *Buchloe dactyloides* (Nutt) Engelm, filter strips retained dissolved phase atrazine and metolachlor to a greater extent than their respective metabolites.[27,28] Subsequent batch equilibrium sorption experiments denoted enhanced sorption of metolachlor and atrazine to VFS soil compared with cultivated soil.[22,23] In contrast, sorption values for

DOI: 10.1002/ps

N-dealkylated metabolites of atrazine and ionic metabolites of metolachlor were not different between soils.[22,23] To date, the persistence of atrazine and metolachlor metabolites in VFS soil has not been evaluated. These data are required to determine the potential transport of these compounds in VFS soil. Thus the objective of the present experiment was to compare the mineralisation kinetics of atrazine and metolachlor with those of their respective metabolites in VFS and cultivated soil.

2 MATERIALS AND METHODS

2.1 Soil

The VFS and cultivated soil were collected from a 0.61 ha watershed constructed by the USDA-ARS in 1937 at the Blackland Research Center (Temple, TX, USA). The soil at the site is classified as Houston Black clay (very fine, smectitic, thermic Oxyaquic Hapluderts). VFS were established with a mixed stand of buffalo grass and Bermuda grass, *Cynodon dactylon* L., in 1991. Since that date the cultivated fields adjacent to the VFS have been in a corn/sorghum rotation. Soil samples were collected from the top 0–5 cm depth of the VFS and cultivated soil, passed through a 2-mm sieve to remove roots and verdure and stored at 22 (±2)°C for less than 3 weeks prior to initiating experiments. Particle size distribution was determined by the hydrometer method.[29] Organic carbon content was measured by combustion in a medium-temperature induction furnace and corrected for total inorganic carbon.[30,31] Soil pH and cation exchange capacity (CEC) were determined by standard methods.[32,33] Soil data are presented in Table 1.

2.2 Microbial and enzymatic characterisation

Numbers of heterotrophic bacteria, Gram-negative bacteria and fungi were determined for VFS and cultivated soil by serial dilution and spread plating. Soil (5 g) was placed in extraction solution (47.5 ml) containing sodium hexametaphosphate (3.3 mM) and zwittergent detergent (6.0 M) and shaken on a horizontal shaker for 5 min at 280 oscillations min^{-1}.[34] Subsequent dilutions were performed with 0.15 M aqueous sodium chloride. Heterotrophic bacteria were enumerated on 10% tryptic soy agar (TSA) containing cycloheximide (100 mg $litre^{-1}$), and Gram-negative bacteria were enumerated on 10% TSA containing both cycloheximide (100 mg $litre^{-1}$) and crystal violet (5 mg $litre^{-1}$).[35] Fungal numbers were assayed by plating onto potato dextrose agar supplemented with Rose Bengal (33 mg $litre^{-1}$) and streptomycin (30 mg $litre^{-1}$).[36] Media were from Difco (Detroit, MI, USA), while the antibiotics and dyes were from Sigma-Aldrich (St Louis, MO, USA). All plate counts were conducted following incubation at 27 °C for 4 days.

Table 1. Properties of the Houston Black clay under cultivation (CS) and vegetated filter strip (VFS)

Soil	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)	Cation exchange capacity ($C\,mol_c\,kg^{-1}$)	pH
VFS	37.9	31.9	30.2	4.2	67.8	7.6
CS	36.8	29.5	33.7	2.5	62.5	7.6

The community-level physiological profile (CLPP) of the soil microbial populations was determined using BIOLOG EcoPlates™ (BIOLOG, Hayward, CA, USA) containing 31 different compounds as sole carbon sources. Each plate well was inoculated (150 µl) with a 10^{-3} dilution from the microbial enumeration procedure described above.[37] Plates were incubated at 27 °C without shaking. Colour development (absorbance) was measured at 590 nm with a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) every 24 h during the 8 day incubation. Absorbance values were blanked against the initial absorbance of each well at $t = 0$ and any subsequent colour development in the control wells. The data were then normalised, to account for initial differences in inoculum density, by dividing the colour development in each well by the average colour development (AWCD) of all 31 wells.[38] The plate profiles were compared using principal component analysis (PCA; SYSTAT v. 10, SPSS, Chicago, IL, USA) when each plate reached an AWCD of approximately 0.75.[39] Additionally, microbial diversity in each soil was determined from the BIOLOG data, collected at 6 days of incubation, using the Shannon, Shannon evenness, Simpson, McIntosh and McIntosh evenness indices.[40,41] For evenness calculations, wells with absorbance $\geq$0.250 were considered to be positive.[39] Any negative absorbance values were considered to be zero for the purpose of data analysis.[39]

Dehydrogenase and aryl acylamidase activities in the soils were determined. For the dehydrogenase assay, soil samples (2 g oven-dry weight equivalent) were amended with aqueous triphenyl-tetrazolium chloride (30 g $litre^{-1}$, 4 ml) and incubated statically at 37 °C.[21,42] After 24 h the reaction was terminated and triphenyl formazan (TPF) was extracted by adding methanol (12 ml) and shaking the tubes horizontally for 30 min at 280 oscillations min^{-1}. Samples were then centrifuged for 10 min at room temperature and 3000 × *g*. The TPF concentration in the supernatant was determined spectrophotometrically at 485 nm. Aryl acylamidase activity was determined by the method of Zablotowicz *et al*.[43] Briefly, soil (1 g oven-dry weight equivalent) was incubated with 2-nitroacetanilide (2 mM, 4 ml; Lancaster, Windham, NH, USA) in phosphate buffer (0.05 M, pH 8.0). Samples were incubated at 30 °C with shaking at 150 oscillations min^{-1} for 24 h. Production of 2-nitroaniline (NAA) was determined by adding methanol (4 ml) to each sample and extracting NAA, as described above for TPF, prior to reading the supernatant spectrophotometrically at 410 nm.

2.3 Herbicides

Experiments were conducted with a mixture of ^{14}C-*ring*-labelled and technical-grade compounds supplied by Syngenta Crop Protection (Greensboro NC, USA). The specific radioactivities and radiochemical purities of the labelled compounds were: metolachlor, 496 MBq mmol^{-1} and 98.5%; ESA, 446 MBq mmol^{-1} and 98.0%; OA, 129 MBq mmol^{-1} and 94.1%; atrazine, 340 MBq mmol^{-1} and 98.7%; DEA, 59 MBq mmol^{-1} and 96.8%; DIA, 170 MBq mmol^{-1} and 97.3%; HA 303 MBq mmol^{-1} and 96.8%. The chemical purities of the non-labelled compounds were: metolachlor, 98.0%; ESA, 95.7%; OA 99.9%; atrazine, 97.9%; DEA, 94.0%; DIA, 96.0%; HA, 97.0%.

2.4 Mineralisation

Field moist soil (5 g oven-dry weight equivalent) was added to 100-ml biometers and brought to −33 kPa moisture content with deionised water containing a mixture of ^{14}C-*ring*-labelled and technical-grade material. The final concentration of all compounds was 1.25 mg kg^{-1} soil and 1.67 kBq of radioactivity per biometer. Autoclaved soil samples were included as a sterile control. Soils were incubated in the dark at 25 °C for 62 days. Evolution of ^{14}C was measured by periodically removing the $^{14}CO_2$ traps and placing the entire volume of trapping solution (1 ml of 1 M aqueous potassium hydroxide) into scintillation vials containing Ecolite (+) (ICN, Costa Mea, CA, USA) liquid scintillation cocktail (10 ml). Radioactivity in the samples was measured by liquid scintillation spectroscopy (LSS) using a Beckman LS 8000 counter (Beckman Instruments Inc., Fullerton, CA, USA). Replicate aliquots of the [^{14}C-*ring*] standard solutions were counted and the average of the counts was used as a reference to standardise the amount of radioactivity added to each biometer. Cumulative $^{14}CO_2$ data were fitted to the Gompertz growth model using SigmaPlot 5.0 (Systat Software Inc., Point Richmand, CA, USA):

$$Y = a\exp\{-\exp[-k(t - t_i)]\}$$

where a is the plateau or maximum percentage of mineralisation, t_i is the abscissa of the inflection point, k is the mineralisation rate constant and t is time.[44,45]

2.5 Mass balance determination

For the final sampling, biometers were dried to a constant weight (24 h at 80 °C). Soil was manually crushed into uniform particle size, and duplicate samples (0.30 g) were weighed onto Whatman 1 Qualitative filter paper (Whatman Inc., Florham Park, NJ, USA). Samples were combusted in a Packard model 306 oxidiser (Packard Instruments, Chicago, IL, USA), and evolved $^{14}CO_2$ was trapped in scintillation vials containing Carbo-Sorb and Permafluor (1 + 1 by volume, 20 ml; Packard Instruments). Radioactivity was determined by LSS. The amount of $^{14}CO_2$ recovered from the combusted samples was added to the cumulative $^{14}CO_2$ evolved from the terminal time course sampling to determine the mass balance of ^{14}C.

2.6 Statistical analysis

Mineralisation data were fitted to the Gompertz growth model. Estimates for a, k and t_i, which correspond to the maximum percentage mineralised, the rate of mineralisation and the lag phase respectively, were subjected to analysis of variance (ANOVA) for a completely randomised design using SAS with treatments in a factorial arrangement (compound × soil). Contrasts were not orthogonal but were chosen for the objective of the study. To control experiment-wise error, significance of a contrast was evaluated only if the corresponding overall F test was significant ($P < 0.05$). Microbial and enzymatic data were analysed using a two-tailed t test. Microbial, enzymatic and mineralisation experiments were repeated, and after statistical analysis showed no differences between data sets, the data were pooled. Microbial diversity indices were subjected to ANOVA.

3 RESULTS AND DISCUSSION

3.1 Soil microbial parameters

With the exception of total bacteria, culturable populations of the evaluated soil micro-organisms and soil enzymatic activities were greater in the VFS soil than in the cultivated soil (Table 2). Higher dehydrogenase activity indicates greater total microbial activity, while enhanced aryl acylamidase activity has been linked with the degradation of chloroacetamide herbicides.[43] Thus increased levels of enzymatic activity observed in the VFS soil agree with the microbial population data and suggest greater potential for herbicide mineralisation. Others have also reported higher microbial populations, soil enzymatic activity and soil microbial activity in VFS soil than in cultivated soil.[18,21]

Table 2. Average values for total fungi ($Fungi_T$), total bacteria ($Bacteria_T$), Gram-negative bacteria ($Bacteria_{GN}$), triphenyl-tetrazolium chloride dehydrogenase activity (TTC-D) and aryl acylamidase activity (Aryl-A) in vegetated filter strip soil (VFS) and cultivated soil (CS)[a]

Soil	$Fungi_T$ (log(cfu[b] g^{-1}))	$Bacteria_T$ (log(cfu g^{-1}))	$Bacteria_{GN}$ (log(cfu g^{-1}))	TTC-D (nmol g^{-1} h^{-1})	Aryl-A (nmol g^{-1} h^{-1})
VFS	5.40 (±0.14) a	7.74 (±0.08) a	6.12 (±0.08) a	40.4 (±0.1) a	214 (±38) a
CS	5.00 (±0.10) b	7.70 (±0.14) a	5.75 (±0.20) b	26.9 (±0.1) b	118 (±5) b

[a] Values are the mean of six replicates (±SD). Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.
[b] Colony-forming units.

Pest Manag Sci **62**:505–514 (2006)
DOI: 10.1002/ps

Table 3. Microbial community diversity indices for cultivated soil (CS) and vegetated filter strip soil (VFS)[a]

	Diversity index			Evenness index	
Soil	Shannon	Simpson	McIntosh	Shannon	McIntosh
CS	3.14 (±0.11) a	20.45 (±3.02) a	6.21 (±0.65) a	0.980 (±0.009) a	0.974 (±0.007) a
VFS	3.27 (±0.04) b	24.16 (±1.21) b	7.79 (±0.59) b	0.974 (±0.009) a	0.979 (±0.002) a
ANOVA	$P = 0.024$	$P = 0.019$	$P = 0.001$	$P = 0.181$	$P = 0.099$

[a] Values are the mean of six replicates (±SD). Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

In addition to higher microbial numbers and enzymatic activity, the CLPP data demonstrated that the microbial community in the VFS soil had more diverse metabolic capabilities (based on tested substrates) and a different metabolic profile than the microbial community in the cultivated soil. Each of the calculated diversity indices was higher for the VFS soil (Table 3), indicating greater metabolic diversity. The evenness indices were close to unity for both soils, signifying that the microbial populations which metabolised the different sole carbon sources were relatively evenly distributed in each soil, but there was no significant difference between the VFS and cultivated soil. The PCA distinctly grouped the cultivated and VFS soil samples based on their metabolic profiles (Fig. 2). The VFS soil was more strongly correlated with metabolism of L-asparagine, L-serine, D-galactonic acid, D-galacturonic acid, λ-hydroxybutyric acid, D-mannitol, Tween 40 and Tween 80, whereas the cultivated soil was more strongly correlated with metabolism of 2-hydroxybenzoic acid, α-cyclodextrin, β-methyl-D-glucoside, L-threonine, itaconic acid, glycyl-L-glutamic acid, α-ketobutyric acid and D,L-α-glycerol phosphate. The VFS results were less variable, suggesting a more homogeneous spatial distribution of microbial populations in the VFS soil as compared with the cultivated soil.

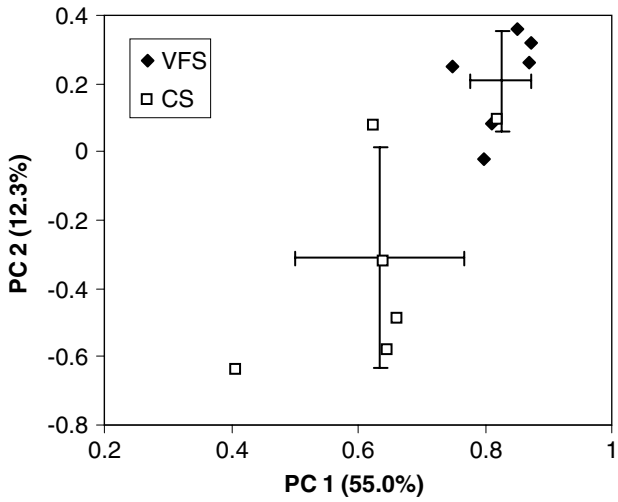

Figure 2. Principal component analysis of community-level physiological profiles for cultivated soil (CS) and vegetated filter strip soil (VFS). Profiles were determined based on the utilisation of 31 different sole carbon sources. Error bars are one standard deviation from the mean of six replicates. The variance represented by each principal component is indicated in parentheses.

3.2 Metolachlor mineralisation

Representative kinetics for the cumulative mineralisation of metolachlor, ESA and OA in VFS and cultivated soil are presented in Fig. 3. With the exception of Kotoula-Syka *et al.*,[46] who reported ≤8% cumulative mineralisation of ^{14}C-*ring*-labelled metolachlor after 52 days of incubation, our data for the cumulative mineralisation of metolachlor in cultivated soil are greater than published results (Table 4). Reported values for the mineralisation of ^{14}C-*ring*-labelled metolachlor in cultivated soil are ≤4% after 46 days of incubation, ≤1.5% after 120 days of incubation and ≤1.6% after 49 days of incubation.[21,47,48] Similarly, our values for

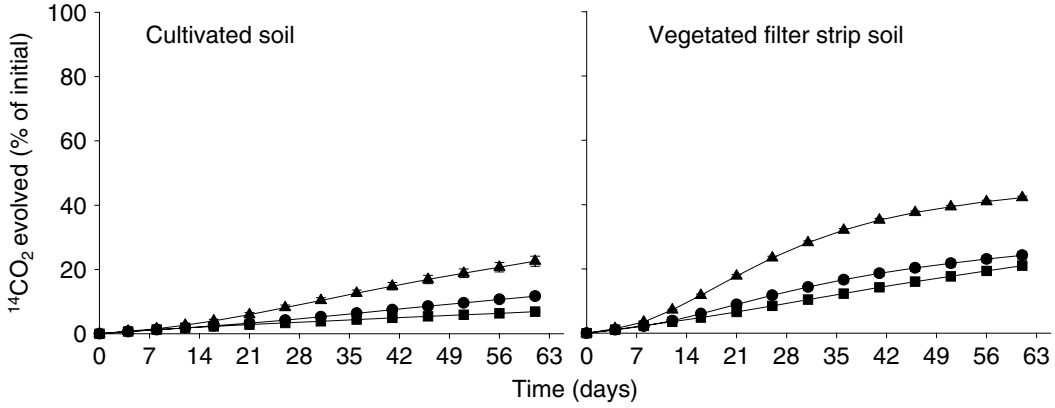

Figure 3. Kinetics of cumulative $^{14}CO_2$ mineralisation for metolachlor (●), metolachlor ethanesulfonic acid (■) and metolachlor oxanilic acid (▲) in cultivated soil and vegetated filter strip soil. Each point is the mean and standard deviation of four replicates. Error bars do not appear when they are smaller than the symbol for the mean. Full lines are fitted values generated from the Gompertz growth model.

Table 4. Average values (±SD) for the initial rate and cumulative mineralisation of metolachlor (MET), metolachlor ethanesulfonic acid (ESA) and metolachlor oxanilic acid (OA) in vegetated filter strip soil (VFS) and cultivated soil (CS). Data were fitted to the Gompertz growth model $Y = a\exp\{-\exp[-k(t - t_i)]\}$, where a is the plateau or maximum percentage of mineralisation, t_i is the abscissa of the inflection point, k is the mineralisation rate constant and t is time

Compound	Soil	a (%)	k (day^{-1})	t_i (days)	Adjusted r^2
MET	VFS	26.4 (±1.0)	0.060 (±0.002)	22.8 (±0.6)	0.999
	CS	17.6 (±1.0)	0.035 (±0.003)	36.8 (±3.1)	0.998
ESA	VFS	27.5 (±0.7)	0.041 (±0.001)	30.4 (±1.0)	0.998
	CS	8.2 (±0.2)	0.042 (±0.002)	23.8 (±0.9)	0.989
OA	VFS	43.6 (±0.5)	0.075 (±0.002)	19.9 (±0.4)	0.999
	CS	29.8 (±2.3)	0.043 (±0.001)	32.5 (±0.9)	0.999

Table 5. *P* values for the maximum percentage of mineralisation (*a*), the mineralisation rate constant (*k*) and the abscissa of the inflection point (t_i)

	Metolachlor and metabolites			Atrazine and metabolites		
Comparison	a	k	t_i	a	k	t_i
Model (*F* test)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Compound	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001
Soil	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Compound × soil	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 6. *P* values for simple effects of compound and soil on the maximum percentage of mineralisation (*a*), the mineralisation rate constant (*k*) and the abscissa of the inflection point (t_i): metolachlor (MET), metolachlor ethanesulfonic acid (ESA), metolachlor oxanilic acid (OA), vegetated filter strip soil (VFS) and cultivated soil (CS)

	a			k			t_i		
Parameter	VFS *vs* CS	MET *vs* ESA	MET *vs* OA	VFS *vs* CS	MET *vs* ESA	MET *vs* OA	VFS *vs* CS	MET *vs* ESA	MET *vs* OA
Compound	–	–	–	–	–	–	–	–	–
MET	0.0001	–	–	0.0001	–	–	0.0001	–	–
ESA	0.0001	–	–	0.3308	–	–	0.0001	–	–
OA	0.0001	–	–	0.0001	–	–	0.0001	–	–
Soil	–	–	–	–	–	–	–	–	–
VFS	–	0.1367	0.0001	–	0.0001	0.0002	–	0.0001	0.0001
CS	–	0.0001	0.0001	–	0.0040	0.0032	–	0.0001	0.0006

the cumulative mineralisation of metolachlor in VFS soil are greater than those of Staddon *et al.*,[21] who reported ≤4.0% cumulative mineralisation of ^{14}C-*ring*-labelled metolachlor after 46 days of incubation. Mineralisation and/or degradation data for ESA and OA have not previously been published.

The kinetic parameters a, k and t_i were significantly different among treatments (Table 5). A compound × soil interaction was detected and simple effects were evaluated (Table 6). For metolachlor and OA, values for a and k were at least 1.5-fold greater and values for t_i were reduced by at least 1.6-fold in the VFS soil. For ESA, values for a were 3.4-fold greater in the VFS soil. However, k was not different between soils and t_i was greater in the VFS soil. These apparent discrepancies are attributed to the low cumulative mineralisation of ESA in the cultivated soil, which resulted in a relatively poor fit to the Gompertz growth model. Mineralisation of metolachlor, ESA and OA in the sterile controls was less than the limit of quantitation (0.6 Bq ml^{-1}) for both soils, indicating that the mineralisation of these compounds is primarily microbially mediated (data not shown).

These data demonstrate that the mineralisation of metolachlor and its primary metabolites, ESA and OA, is enhanced in VFS soil. Others have reported enhanced degradation of metolachlor in VFS soil compared with cultivated soil.[21,24,25] Thus our data not only corroborate published results which indicate that the persistence of metolachlor is reduced in VFS soil, but also indicate that the persistence of the ionic metabolites of metolachlor, ESA and OA, will likely be reduced in VFS owing to enhanced mineralisation.

Since the transport of herbicides in soil is intimately linked with their persistence and mobility, comparing the mineralisation data for these compounds with previously published sorption data provides an insight into the potential transport of these compounds from VFS. Krutz *et al.*[22] reported K_d values for the sorption of metolachlor, ESA and OA to Houston Black clay under cultivation and VFS. K_d values for metolachlor were 8.3 litre kg^{-1} for VFS soil and 4.4 litre kg^{-1} for

DOI: 10.1002/ps

cultivated soil. In contrast, K_d values for ESA and OA were approximately 0.8 litre kg^{-1} for both soils. Krutz *et al.*[22] suggested that the potential mobility of metolachlor is reduced in VFS soil owing to the compound's affinity for soil organic carbon. In contrast, the mobility of the ionised metabolites of metolachlor would likely be similar between soils, since factors other than soil organic carbon control their sorption. Consequently, for VFS to effectively reduce the transport of the ionised metabolites, their persistence must be reduced relative to that in adjacent cultivated soil. These data indicate that the persistence of metolachlor, ESA and OA is reduced in VFS soil. Therefore the transport of metolachlor, ESA and OA may be curtailed in VFS soil relative to cultivated soil.

3.3 Atrazine mineralisation

Representative kinetics for the cumulative mineralisation of atrazine, DEA, DIA and HA in VFS and cultivated soil are presented in Fig. 4. The kinetic parameters a, k and t_i were significantly different among treatments (Table 5). A compound × soil interaction was detected and simple effects were evaluated (Table 7). For atrazine, DEA and HA, t_i values were at least 1.5-fold higher in the VFS soil than in the cultivated soil, while values for a and k were at least 1.7-fold higher in the cultivated soil (Table 8). In contrast, t_i and k values for DIA were not different between soils, and values for a were 1.3-fold higher in the VFS soil.

Mineralisation of atrazine and its metabolites in the sterile controls was less than the limit of quantitation (0.6 Bq ml^{-1}) for both soils, indicating that the mineralisation of these compounds is primarily microbially mediated (data not shown).

With the exception of DIA, these data demonstrate that the mineralisation of atrazine and its primary metabolites was reduced in the VFS soil compared with the cultivated soil. This implies that the persistence of atrazine and its metabolites may be greater in VFS soil than in cultivated soil owing to enhanced mineralisation in the latter.

Although our data for enhanced mineralisation of atrazine and its metabolites in cultivated soil are counterintuitive, similar observations have been noted in the literature. Reungsang *et al.*[49] observed greater mineralisation of ^{14}C-*ring*-labelled atrazine in cultivated soil (60%) compared with 3- (1% mineralised), 5- (12% mineralised) and 9-year-old (2% mineralised) filter strips established in switchgrass (*Panicum virgatum* L.). Additionally, Ostrofsky *et al.*[50] reported enhanced mineralisation of atrazine in

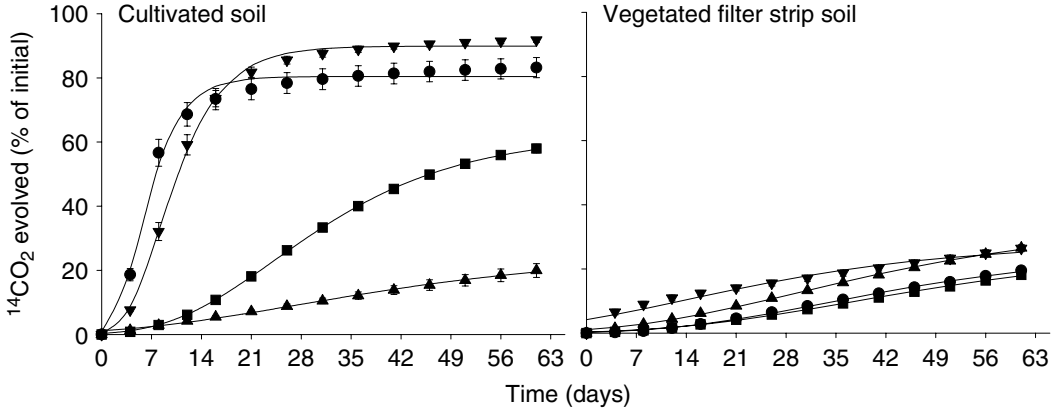

Figure 4. Kinetics of cumulative $^{14}CO_2$ mineralisation for atrazine (●), desethylatrazine (■), deisopropylatrazine (▲) and hydroxyatrazine (▼) in cultivated soil and vegetated filter strip soil. Each point is the mean and standard deviation of four replicates. Error bars do not appear when they are smaller than the symbol for the mean. Full lines are fitted values generated from the Gompertz growth model.

Table 7. *P* values for simple effects of compound and soil on the maximum percentage of mineralisation (*a*), the mineralisation rate constant (*k*) and the abscissa of the inflection point (t_i): atrazine (ATR), desethylatrazine (DEA), deisopropylatrazine (DIA), hydroxyatrazine (HA), vegetated filter strip soil (VFS) and cultivated soil (CS)

	a				*k*				t_i			
Parameter	VFS *vs* CS	ATR *vs* DEA	ATR *vs* DIA	ATR *vs* HA	VFS *vs* CS	ATR *vs* DEA	ATR *vs* DIA	ATR *vs* HA	VFS *vs* CS	ATR *vs* DEA	ATR *vs* DIA	ATR *vs* HA
Compound	–	–	–	–	–	–	–	–	–	–	–	–
ATR	0.0001	–	–	–	0.0001	–	–	–	0.0001	–	–	–
DEA	0.0001	–	–	–	0.0001	–	–	–	0.0001	–	–	–
DIA	0.0001	–	–	–	0.8502	–	–	–	0.0065	–	–	–
HA	0.0001	–	–	–	0.0001	–	–	–	0.0001	–	–	–
Soil	–	–	–	–	–	–	–	–	–	–	–	–
VFS	–	0.4630	0.0001	0.0514	–	0.2071	0.4617	0.9606	–	0.0001	0.0001	0.0001
CS	–	0.0001	0.0001	0.0001	–	0.0001	0.0001	0.0001	–	0.0001	0.0001	0.0002

Table 8. Parameters of atrazine (ATR), desethylatrazine (DEA), deisopropylatrazine (DIA) and hydroxyatrazine (HA) mineralisation kinetics obtained for vegetated filter strip soil (VFS) and cultivated soil (CS) after fitting to the Gompertz growth model; *a* indicates the maximum percentage of mineralisation, *k* the mineralisation rate constant and t_i the abscissa of the inflection point

Compound	Soil	*a* (%) (±SD)	*k* (day^{-1}) (±SD)	t_i (days) (±SD)	Adjusted r^2
ATR	VFS	25.4 (±0.3)	0.046 (±0.001)	33.6 (±0.9)	0.998
	CS	80.4 (±3.1)	0.297 (±0.016)	5.1 (±0.2)	0.990
DEA	VFS	26.4 (±0.6)	0.039 (±0.001)	37.3 (±1.4)	0.998
	CS	62.5 (±1.9)	0.068 (±0.002)	24.2 (±0.7)	0.999
DIA	VFS	32.9 (±1.9)	0.042 (±0.001)	29.6 (±0.7)	0.998
	CS	25.1 (±0.5)	0.040 (±0.001)	27.5 (±0.3)	0.994
HA	VFS	28.2 (±1.1)	0.046 (±0.002)	14.1 (±1.6)	0.955
	CS	89.9 (±0.8)	0.206 (±0.010)	8.1 (±0.3)	0.998

cultivated soil compared with an adjacent riparian soil.

Enhanced mineralisation of atrazine in the cultivated soil is likely due to repeated applications of atrazine facilitating the development of a microbial population capable of rapidly mineralising the compound. Greater atrazine degrader populations and *s*-triazine ring cleavage genes have been reported for cultivated soils that receive repeated applications of atrazine compared with adjacent VFS soil and riparian soils that do not receive applications of atrazine.[49,50] The implication is that the persistence of atrazine and many of its metabolites may be greater in VFS soil than in cultivated soil when an active degrader population is present in the latter. Additionally, the kinetics of hydroxyatrazine mineralisation in the VFS soil were similar to those for atrazine mineralisation, which suggests that the primary pathway of atrazine mineralisation in the VFS soil was through a hydroxyatrazine intermediate. This degradation pathway has been found in numerous bacteria isolated from atrazine-exposed sites around the world.[51,52] This growing body of work indicates that weed management practices may impact the relative ability of VFS to reduce the transport of atrazine and its metabolites relative to adjacent cultivated soils.

Ideally, for the transport of atrazine and its metabolites to be reduced in VFS soil, estimates for mobility and persistence should be lower in VFS soil relative to cultivated soil. Krutz *et al.*[23] observed greater sorption of atrazine and HA to Houston Black clay under VFS compared with adjacent Houston Black clay under cultivation. They concluded that the potential mobility of these compounds is reduced in the former. In contrast, Krutz *et al.*[23] observed similar sorption characteristics for DEA and DIA between VFS soil and cultivated soil, concluding that their mobility would likely be similar between soils. In the present study, mineralisation data indicate that the persistence of atrazine, DEA and HA is greater in VFS soil owing to reduced mineralisation. Thus, for the potential transport of atrazine, DEA and HA to be curtailed in VFS, enhanced sorption of these compounds to the VFS soil must offset prolonged persistence.

The mass balance of ^{14}C recovered ranged from 93.4 to 100.3% for atrazine, DEA, DIA and HA in sterile and non-sterile VFS and cultivated soil (average SD ±3.73). For metolachlor, ESA and OA the mass balance of ^{14}C recovered ranged from 80.4 to 89.2% in sterile and non-sterile VFS and cultivated soil (average SD ±2.45). Lower ^{14}C recovery of metolachlor and its metabolites may be associated with volatilisation, which can be an important dissipation mechanism for metolachlor, ranging from 6 to 22% in one field study, with greater losses in plant residue-managed soils.[53] Metolachlor, ESA and OA volatilisation was not measured in the present study but may account for some of the discrepancy in ^{14}C recovery.[21]

4 CONCLUSION

In summary, our results corroborate previous studies regarding the persistence of metolachlor in VFS soil relative to cultivated soil. Under the conditions in this study we have further determined that the persistence of the ionic metabolites of metolachlor, ESA and OA, is reduced in VFS soil compared with cultivated soil. The implication is that VFS may curtail the transport of metolachlor, ESA and OA relative to adjacent cultivated soil owing to enhanced mineralisation in the former. In contrast to the behaviour of metolachlor and its ionic metabolites, the mineralisation of atrazine was greater in the cultivated soil. Others have reported similar observations, suggesting that repeated applications of atrazine to cultivated soil facilitate the development of an atrazine degrader population(s). In addition, we observed greater mineralisation of DEA and HA in the cultivated soil. These data indicate that the potential transport of atrazine and its metabolites may actually be greater in VFS soil than in cultivated soil when an active degrader population is present in the cultivated soil and the longer persistence in the VFS is not offset by increased sorption.

REFERENCES

1 Vencill WK (ed.), *Herbicide Handbook* (8th edn). Weed Science Society of America, Champaign, IL (2002).

2 Muir D and Baker B, The disappearance and movement of three triazine herbicides and several of their degradation products in soil under field conditions. *Weed Res* **18**:111–120 (1978).

3 Behki R and Khan S, Degradation of atrazine by *Pseudomonas*: *N*-dealkylation and dehalogenation of atrazine and its metabolites. *J Agric Food Chem* **34**:746–749 (1986).
4 Behki R and Khan S, Degradation of atrazine, propazine and simazine by *Rhodococcus* strain B-30. *J Agric Food Chem* **42**:1237–1241 (1994).
5 Mandelbaum R, Wackett L and Allan D, Rapid hydrolysis of atrazine to hydroxyatrazine by soil bacteria. *Environ Sci Technol* **27**:1943–1946 (1993).
6 Armstrong D, Chesters G and Harris R, Adsorption catalyzed chemical hydrolysis of atrazine. *Environ Sci Technol* **31**:61–66 (1968).
7 Hostetler KA and Thurman EM, Determination of chloroacetanilide herbicide metabolites in water using high-performance liquid chromatography–diode array detection and high-performance liquid chromatography/mass spectrometry. *Sci Total Environ* **248**:147–155 (2000).
8 Phillips PJ, Wall GR, Thurman EM, Eckhardt DA and Vanhoesen J, Metolachlor and its metabolites in tile drain and stream runoff in the Canajoharie Creek watershed. *Environ Sci Technol* **33**:3531–3537 (1999).
9 Yokley RA, Mayer LC, Huang SB and Vargo JD, Analytical method for the determination of metolachlor, acetochlor, alachlor, dimethenamid and their corresponding ethanesulfonic and oxanilic acid degradates in water using SPE and LC/ESI-MS/MS. *Anal Chem* **74**:3754–3759 (2002).
10 Aga DS, Thurman EM, Yockel ME, Zimmerman LR and Williams TD, Identification of a new sulfonic acid metabolite of metolachlor in soil. *Environ Sci Technol* **30**:592–597 (1996).
11 Field JA and Thurman EM, Glutathione conjugation and contaminant transformation. *Environ Sci Technol* **30**:1413–1418 (1996).
12 Lerch RN, Blanchard PE and Thurman EM, Contribution of hydroxylated atrazine degradation products to the total atrazine load in Midwestern streams. *Environ Sci Technol* **32**:40–48 (1998).
13 Clark GM and Goolsby DA, Occurrence and load of selected herbicides and metabolites in the lower Mississippi River. *Sci Total Environ* **248**:101–113 (2000).
14 Pereira W and Hostettler F, Nonpoint source contamination of the Mississippi River and its tributaries by herbicides. *Environ Sci Technol* **27**:1542–1552 (1993).
15 Scribner EA, Battaglin WA, Goolsby DA and Thurman EM, Changes in herbicide concentrations in Midwestern streams in relation to changes in use, 1989–1998. *Sci Total Environ* **248**:255–263 (2000).
16 Thurman EM, Bastian KC and Mollhagen L, Occurrence of cotton herbicides and insecticides in playa lakes of the High Plains of West Texas. *Sci Total Environ* **248**:189–200 (2000).
17 Kalkhoff SJ, Kolpin DW, Thurman EM, Ferrer I and Barcelo D, Degradation of chloroacetanilide herbicides: the prevalence of sulfonic and oxanilic acid metabolites in Iowa groundwaters and surface waters. *Environ Sci Technol* **32**:1738–1740 (1998).
18 Benoit P, Barriuso E, Vidon P and Real B, Isoproturon sorption and degradation in a soil from grassed buffer strip. *J Environ Qual* **28**:121–129 (1999).
19 Blanche SB, Shaw DR, Massey JH, Boyette M and Smith MC, Fluometuron adsorption to vegetative filter strip components. *Weed Sci* **51**:125–129 (2003).
20 Rankins Jr A, Shaw DR and Kingery WL, Comparison of fluometuron sorption to soil from a filter strip and cropped field. *Weed Sci* **50**:820–823 (2002).
21 Staddon WJ, Locke MA and Zablotowicz RM, Microbiological characteristics of a vegetative buffer strip soil and degradation and sorption of metolachlor. *Soil Sci Soc Am J* **65**:1136–1142 (2001).
22 Krutz LJ, Senseman SA, McInnes KJ, Hoffman DW and Tierney DP, Adsorption and desorption of metolachlor and metolachlor metabolites in vegetated filter strip and cultivated soil. *J Environ Qual* **33**:939–945 (2004).
23 Krutz LJ, Senseman SA, McInnes KJ, Zuberer DA and Tierney DP, Adsorption and desorption of atrazine, desethylatrazine, deisopropylatrazine and hydroxyatrazine in vegetated filter strip and cultivated soil. *J Agric Food Chem* **51**:7379–7384 (2003).
24 Mersie W, Seybold CA, McNamee C and Huang J, Effectiveness of switchgrass filter strips in removing dissolved atrazine and metolachlor from runoff. *J Environ Qual* **8**:816–821 (1999).
25 Seybold C, Mersie W and Delorem D, Removal and degradation of atrazine and metolachlor by vegetative filter strips on clay loam soil. *Commun Soil Sci Plant Anal* **32**:723–737 (2001).
26 Shankle MW, Shaw DR and Boyette M, Confirmation of an enzyme-linked immunosorbent assay to detect fluometuron in soil. *Weed Technol* **15**:669–675 (2001).
27 Krutz LJ, Senseman SA, Dozier MC, Hoffman DW and Tierney DP, Infiltration and adsorption of dissolved atrazine and atrazine metabolites in buffalograss filter strips. *J Environ Qual* **32**:2319–2324 (2003).
28 Krutz LJ, Senseman SA, Dozier MC, Hoffman DW and Tierney DP, Infiltration and adsorption of dissolved metolachlor, metolachlor oxanilic acid, and metolachlor ethanesulfonic acid by buffalograss (*Buchloe dactyloides*) filter strips. *Weed Sci* **52**:166–171 (2004).
29 Bouyoucos GJ, An improved type of soil hydrometer. *Soil Sci* **76**:377–378 (1953).
30 Allison LE, Bollen WB and Moodie CD, Total carbon, in *Methods of Soil Analysis, Part 2*, ed. by Black CA. Soil Science Society of America, Madison, WI, pp. 1353–1361 (1965).
31 Dremanis A, Quantitative gasometric determination of calcite and dolomite by using the Chittick apparatus. *J Sedim Petrol* **32**:520–529 (1962).
32 Thomas GW, Soil pH and soil acidity, in *Methods of Soil Analysis, Part 3, Chemical Methods*, ed. by Sparks DL. Soil Science Society of America, Madison, WI, pp. 475–490 (1996).
33 Soil Survey Laboratory Staff, Soil survey laboratory methods manual. *Soil Surv Invest Rep 42*, USDA-SCS, Washington, DC (1992).
34 Brendecke JW, Axelson RD and Pepper IL, Soil microbial activity as an indicator of soil fertility; the long term effects of municipal sewage sludge on an arid soil. *Soil Biol Biochem* **25**:751–758 (1993).
35 Gould WD, Hagedorn C, Bardinelli TR and Zablotowicz RM, New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. *Appl Environ Microbiol* **49**:28–32 (1985).
36 Martin JP, Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci* **69**:215–232 (1950).
37 Müller AK, Rasmussen LD and Sørensen SJ, Adaptation of the bacterial community to mercury contamination. *FEMS Microbiol Lett* **204**:49–53 (2001).
38 Garland JL and Mills AL, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl Environ Microbiol* **57**:2351–2359 (1991).
39 Garland JL, Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biol Biochem* **28**:213–221 (1996).
40 Magurren AE, *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, NJ (1988).
41 Staddon WJ, Duchesen LC and Trevors JT, Microbial diversity and community structure of post-disturbance forest soils as determined by sole-carbon-source utilization patterns. *Microb Ecol* **34**:125–130 (1997).
42 Casida LE, Klein Jr DA and Santoro T, Soil dehydrogenase activity. *Soil Sci* **98**:371–376 (1964).
43 Zablotowicz RM, Hoagland RE and Wagner SC, 2-Nitroacetanilide as substrate for determination of aryl acylamidase activity in soils. *Soil Biol Biochem* **30**:679–686 (1998).

44 Piutti S, Hallet S, Rousseaux S, Philippot L, Soulas S and Martin-Laurent F, Accelerated mineralisation of atrazine in maize rhizosphere soil. *Biol Fert Soils* **36**:434–441 (2002).
45 Soulas G, Evidence for the existence of different physiological groups in the microbial community responsible for 2,4-D mineralization in soil. *Soil Biol Biochem* **4**:443–449 (1993).
46 Kotoula-Syka E, Hatzios KK, Berry DF and Wilson HP, Degradation of acetanilide herbicides in history and non-history soils from Eastern Virginia. *Weed Technol* **11**:403–409 (1997).
47 Rice PJ, Anderson TA and Coats JR, Degradation and persistence of metolachlor in soil: effects of concentration, soil moisture, soil depth and sterilization. *Environ Toxicol Chem* **21**:2640–2648 (2002).
48 Miller JL, Wollum III AG and Weber JB, Degradation of C^{14}-atrazine and C^{14}-metolachlor in soil from four depths. *J Environ Qual* **26**:633–638 (1997).
49 Reungsang A, Moorman TB and Kanwar RS, Transport and fate of atrazine in Midwestern riparian buffer strips. *J Am Water Res Assoc* **37**:1681–1692 (2001).
50 Ostrofsky EB, Robinson JB, Traina SJ and Tuovinen OH, Effect of cyanuric acid amendment on atrazine mineralization in surface soils and detection of the s-triazine ring-cleavage gene *trz*D. *Soil Biol Biochem* **33**:1539–1545 (2001).
51 De Souza ML, Seffernick J, Martinez B, Sadowsky MJ and Wackett LP, The atrazine catabolism genes *atzABC* are widespread and highly conserved. *Appl Environ Microbiol* **180**:1951–1954 (1998).
52 Topp E, Zhu H, Nour SM, Houot S, Lewis M and Cuppels D, Characterization of an atrazine-degrading *Pseudoaminobacter* sp. isolated from Canadian and French agricultural soils. *Appl Environ Microbiol* **66**:2773–2782 (2000).
53 Prueger JH, Hatfield JL and Sauer TJ, Field-scale metolachlor volatilization flux estimates from broadcast and banded application methods in Central Iowa. *J Environ Qual* **28**:75–81 (1999).

Pest Manag Sci **62**:505–514 (2006)
DOI: 10.1002/ps